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# POSTNATAL ALTERATIONS IN DEVELOPMENT RESULTING FROM PRENATAL EXPOSURE TO PESTICIDES

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## ABSTRACT

Alterations in the developmental processes of embryos resulting from exposure to chemicals are not limited to morphological abnormalities that can be observed in the near term fetus. In our research on the developmental toxicology of pesticides we have noted morphological defects (cataracts, ablation of Harderian gland development, malformation of the otoliths) in postnatal offspring exposed prenatally that could not be detected in fetal examinations because of the immature nature of these structures at that stage of developement. In addition, other effects on physiological functions have been detected (locomotor hyperactivity, and decreased renal concentrating ability) that have no obvious morphological basis. Thus, a postnatal component of a standard teratology bioassay can yield critical new information in the overall assessment of developmental toxicity.

## INTRODUCTION

In the assessment of developmental toxicity, four major endpoints of adverse effects are considered: 1) death of the conceptus, 2) structural abnormalities such as cleft palate, 3) altered growth, such as fetal growth retardation, and 4) functional deficiencies of organ systems (ref. 1). Generally accepted guidelines for the assessment of teratogenic potential (e.g. the FIFRA Guidelines) basically address only the first three manifestations of developmental toxicity. The fourth currently recieves little attention in such studies because their focus is on the morphology of the fetus rather than its physiology; in addition, many organ systems are not yet functional (e.g. the lungs and reproductive organs) or fully differentiated (e.g. the central nervous system, the reproductive tract, and the excretory system) in the fetal rodent. Yet it has become increasingly clear over the last decade that subtle alterations in the performance of a variety of key organ systems may result from exposure during critical prenatal periods. Much of the early work in this field occurred in the area of central nervous system dysfunction, and the study of 'behavioral teratology' has been widespread since the mid 1970's. That pesticides may induce such changes is exemplified by the use of methyl mercury as a prototype behavioral teratogen (ref. 2). Less work has been done on the effects of in utero exposure to chemicals on the function of other organ systems, but sufficient information exists to indicate that the cardiopulmonary, immune, endocrine, hepatic, renal and reproductive systems are also subject to alterations in functional competence. Oftentimes these functional changes are seen at dose levels below those inducing overt structural malformations. The following examples of

such observations have been derived from work performed in our laboratory on approaches to evaluating such effects in the offspring of rodents exposed to xenobiotics in utero.

## NITROFEN INDUCED DEVELOPMENTAL TOXICITY

Nitrofen (2,4-dichloro-p-nitrophenyl ether) is a herbicide with extremely low toxicity to adult mammals, yet has a remarkable ability to adversely affect the development of embryos. While extensive research has indicated that the most sensitive measure of nitrofen teratogenicity are the diaphragmatic hernias (which occur at doses of less than 1 mg/kg/d), a variety of effects have been observed at slightly higher dose levels. Some of the nitrofen related effects occur in tissues and organ systems that are extremely difficult to evaluate in the rodent fetus. Even the magnitiude of neonatal mortality following in utero exposure to nitrofen cannot be predicted by routine methodologies, primarily due to its impact on cardiopulmonary development and function (refs. 3 and 4). Other effects (e.g. ablation of Harderian gland development) do not become obvious until later in development (refs. 5 and 6). The Harderian gland is an exocrine gland located in the orbit of reptiles birds and mammals that have a nictitating membrane. This teratogenic effect is not identifiable in the fetus because the glands are small and inactive until the animals are 10-12 days old. At that time the gland grows rapidly and begins its secretory process under the partial influence of thyroid gland. Other nitrofen-induced effects that become evident in the postnatal period include altered renal function (refs. 7 and 8), hyperactivity (refs. 9 and 10), and altered organ growth (ref. 9). In renal function studies, pups exposed to 75 mg/kg on day 11 of gestation had a diminished

capacity to concentrate their urine regardless of the presence or absence of hydronephrosis. Although only offspring with hydronephrosis had altered renal function after the neonatal period, the techniques applied to the neonates were predictive of long term alterations in renal physiology. Delayed development of the renal papilla is a relatively common finding in teratology studies, and the procedures used in this study may provide an efficient means of determining whether the delay is a transient developmental phenomenom, or whether progressive damage to renal parenchyma is likely to occur.

## DINOCAP INDUCED TORTICOLLIS

Dinocap (a mixture of 2,4- and 2,6- dinitrooctylphenyl crotonates) is a fungicide used in the control of powdery mildew on a variety of crops. Using standard teratology bioassay techniques, we have demonstrated that exposure of mice to dinocap during organogenesis induces fetal growth retardation, cleft palate and supernumerary ribs in the fetus at doses well below those associated with maternal toxicity (ref. 12). While fetal growth was inhibited at the lowest dose tested (5 mg/kg/d), malformations were seen only at 20 mg/kg/d and higher. When other dams were allowed to litter and their offspring evaluated during postnatal maturation, a peculiar behavioral abnormality was observed in the home cage (ref. 12). After postnatal day 21 a number of mice exposed to 12 and 25 mg/kg/d dinocap displayed a twisting of the neck with resultant head tilt which we referred to as torticollis. These mice held their heads tilted constantly to one side. As these animals aged, the behavior of some became more aberrant; some circled repeatedly in one direction, others bobbed

their heads and did backflips, while others would roll over constantly. At 30 days of age, the incidence of affected individuals was 6% at 12 mg/kg/d and 24% at 25 mg/kg/d. (Similar effects were not observed in either the rat or the hamster). This particular behavior has been observed previously in a number of mutant strains of mice and in offspring of manganese deficient mice. In those two examples, the behavioral deficit has been attributed to defects in the development of the otoliths of the inner ear. Recently (ref. 13) we have demonstrated a similar etiology for the dinocap induced torticollis. The otoliths of the mammalian ear are gravity receptors located in the utricular and sacular regions and are responsible for sensing static equilibrium and linear acceleration. Each is composed of a sensory epithelium of hair cells over which lies the otoconial membrane. The membrane itself is composed of crystals of calcium carbonate (the otoconia) embedded in a mucopolysacchride matrix. The examination of these very small, highly specialized sense organs requires a particular dissection and clearing procedure which is not typical of a standard fetal skeletal examination. While malformations of the otoliths may be difficult to routinely detect in the fetus, observations of home cage behaviors can readily identify the lesion. Similar to the example with the herbicide nitrofen, the utility of a postnatal component of the standard teratology examination is clearly demonstrated in the effects of dinocap on the embryonic mouse. Unlike the example of nitrofen, however, the dinocap induced functional effects are detected at a dose level below that which causes defects that can be detected by standard procedures.

## ENDRIN INDUCED HYPERACTIVITY

Endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a,-octahydro-1,4-endo-5,8-dimethanonapthalene) is one of the few cyclodiene insecticides still registered for use in United States. In teratology studies (ref. 14), endrin induced fetal growth retardation in the mouse, but not the rat, at dose levels that were also toxic to the dams. In the hamster (ref. 15), exposure to endrin during the entire period of organogenesis resulted in incresaed prenatal mortality and fetal growth retardation at maternally toxic doses (1.5 mg/kg/d). However, when exposure was limited to a single dose on day 8 of gestation, fused ribs and meningoencephaloceles were observed at a nonmaternally toxic dose (5 mg/kg). In a subsequent study, we followed the behavioral development of rats and hamsters exposed perinatally to endrin (ref. 16). Open field behaviors and Figure 8 maze activities were recorded on multiple days between the onset of activity (postnatal day 15) and adulthood. The Figure 8 maze is an automated activity monitor that records the photocell interuptions which occur as an animal travels through the alleys. Because it is an automated device, longer term observations of activity are possible using this apparatus. In the hamster, exposure to 1.5 mg/kg/d on days 5-14 of gestation produced a persistent elevation in postnatal locomotor activity when measured either in an open field or the Figure 8 maze. The increase in activity amounted to 75% on day 15, 45% on day 20 and 23% at 125 days of age. Nonlocomotor behaviors, such as rearing, running wheel activity and sexual behaviors were unaffected by endrin treatment, indicating the specificity of the behavioral effect. Rats exposed to 0.15 or 0.30 mg/kg/d from time of implantation to beginning of solid food intake (day 16 of lactation) were 30% more active than controls prior to weaning, but

not as adults. These doses did not result in maternal toxicity or affect postnatal growth or viability. The similarity of behavioral changes noted in the young of both species is suggestive of similar alterations of the central nervous system function even though endrin produces morphological defects only in the hamster.

## MIREX INDUCED CATARACTS

Mirex (dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta(c,d)-pentalene) is a pesticide once used extensively for fire ant control in the southeastern regions of the U.S. That mirex could cause cataracts in developing rodents was first noted in pups nursing mothers exposed to the pesticide (refs. 17 and 18). Subsequently it has been demonstrated that cataracts also are present in fetuses exposed transplacentally (refs. 19-20). Similar to the case with nitrofen induced ablation of Harderian gland development and dinocap induced malformations of the otoliths, the cataracts in the fetus were difficult to detect. Not only are the routine fixation and examnination procedures not well suited for detecting cataracts in the fetus, but the cataracts occcured in edematous fetuses, thus possibly obscuring the more subtle effect. A systemic hypoproteinemia was correlated with the formation of cararacts, once again pointing to alterations in perinatal physiology as an important toxicological endpoint. While relatively sophisticated techniques are necessary to pinpoint the etiology of this lesion, the adverse outcome of chemical exposure during the developmental period manifested itself in an obvious fashion when the postnatal growth and development of the offspring was monitored.

## CONCLUSION

The examples presented above clearly demonstrate that teratogenic effects which may seriously influence the quality of life of the affected individuals can go undetected in standard teratology bioassays. Some of these effects might be expected to be detected in a well designed and executed multigeneration study, but are more likely to be detected in a teratology-type bioassay due to the usually higher dosages, route of administration (oral gavage versus dietary), as well as shorter duration of treatment (less chance for induction of detoxification pathways). However, in order to take advantage of the factors of the teratology bioassay that favor the induction of adverse developmental outcomes, it is necessary to extend the period of observation into the postnatal period. Once the developmental effects are observed, further experiments can be designed to fully characterize the nature of the effect and their position on the overall dose-response curve. The types of observations to include in the initial investigation need not be highly sophisiticated because many of the lesions that can be missed in the standard fetal visceral and skeletal examinations express themselves quite visibly when the appropriate phase of development is reached.

DISCLAIMER

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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